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Review

Multiple roles of the invariant chain in MHC class II function

Pamela Stumptner-Cuvelette, Philippe Benaroch *

INSERM U 520, Institut Curie, 12 rue Lhomond, 75005 Paris, France

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1. Introduction

Major histocompatibility complex class II molecules (MHC II) have developed a particular trafficking pathway, which allows them to load antigenic peptides generated in the endosomal/lysosomal system. Following the original description of MHC II associated invariant chain (Ii) in 1983 [1,2], evidence has accumulated demonstrating that its binding to MHC II α and β chains is crucial at several steps of MHC II biogenesis and function [3]. Despite the small size of Ii, this protein comprises an impressive and still growing number of functions (see Table 1). Here, we will review the different roles of Ii, while following the journey of nascent MHC II complexes through the cell. Special attention will be given to the molecular events taking place in the endoplasmic reticulum (ER), during the transport to and within the endocytic pathway.

Newly synthesized MHC II α and β chains associate with Ii in the ER. The resulting nonameric complexes $(\alpha\beta Ii)_3$ exit from the ER, traffic through the Golgi and are then directed towards the endocytic pathway. There, Ii is progressively degraded until only an Ii derived peptide called CLIP (for CLass II associated Invariant chain derived Peptides) remains associated with MHC II. CLIP is then exchanged for an antigenic peptide in a reaction catalyzed by the non-classical MHC II, human leukocyte antigen (HLA)-DM and -DO in human (H2-DM and -DO in mouse according to the new nomenclature [4]). The resulting mature MHC II–peptide complexes are ultimately released to the cell surface for recognition by $CD4^+$ Th cells.

2. Within the endoplasmic reticulum

2.1. Structure of the Ii chain

Ii chain is a non-polymorphic type II integral membrane protein, where the N-terminus constitutes a short cytoplasmic tail of 28 amino acids (aa) (Fig. 1). Alternative initiation of translation and differential splicing of the transcription products generate four different isoforms in human (p33, p35, p41 and p43) [5]. Because of its predominance we will mainly focus on the 216 aa long isoform, which is referred to as p33 in human (and often termed p31 in mouse) according to its apparent molecular mass [1].

Ii assembles to homotrimers immediately after syn-

Abbreviations: aa, amino acid(s); AEP, asparaginyl endopeptidase; APC, antigen presenting cell; CCV, clathrin coated vesicle; CLIP, class II invariant chain associated peptide; DC, dendritic cell; EE, early endosome(s); ER, endoplasmic reticulum; HEL, hen egg lysozyme; HLA, human leukocyte antigen; Ii, invariant chain; MHC II, major histocompatibility complex class II molecule(s); MVB, multivesicular bodies; MIIC, MHC class II rich compartment; M6PR, mannose 6-phosphate receptor; TGN, trans-Golgi network; TfR, transferrin receptor

* Corresponding author. Fax: +33-1-42-34-64-38.
E-mail address: benaroch@curie.fr (P. Benaroch).

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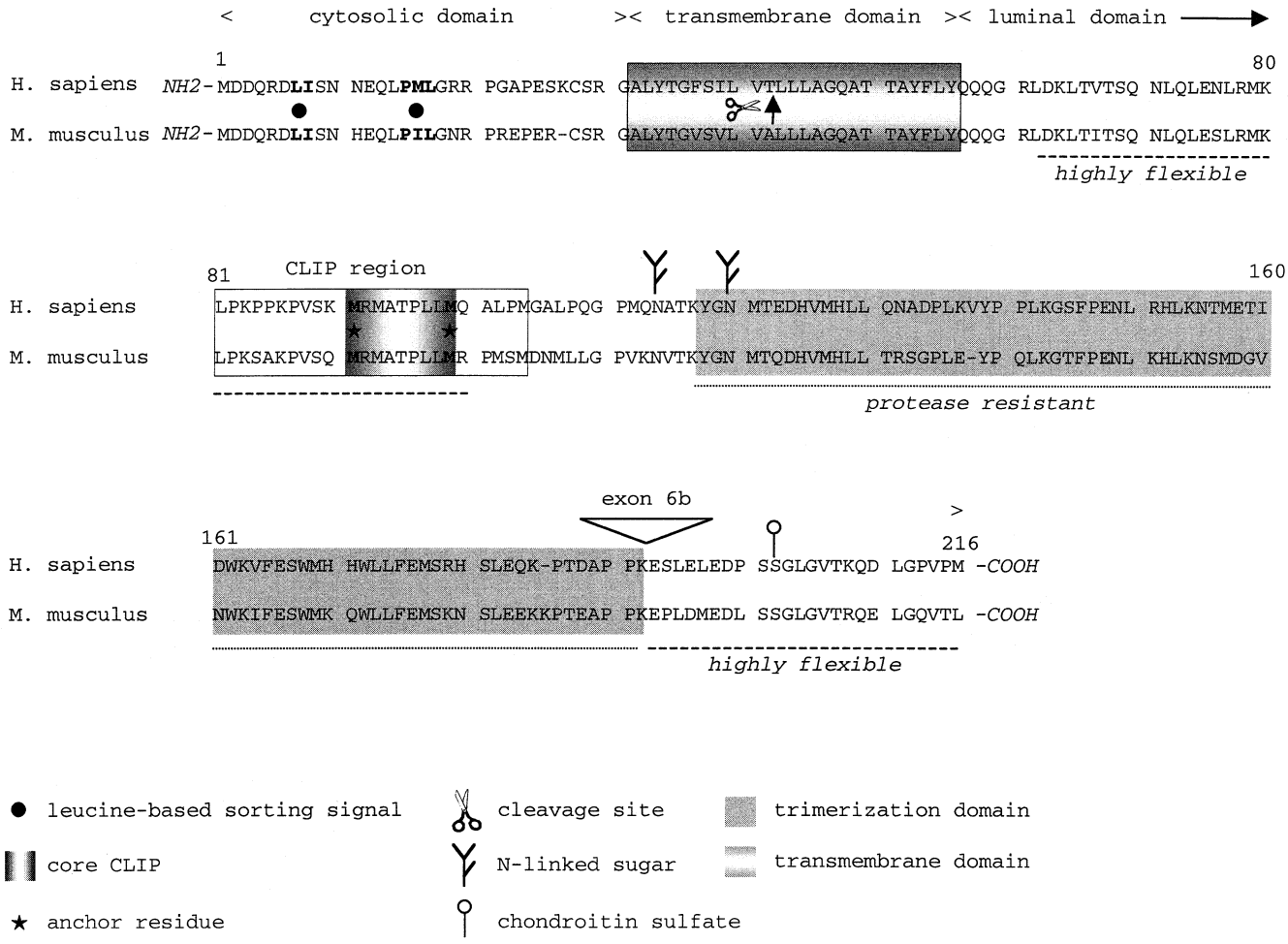

Fig. 1. Structural features of the p33 invariant chain. An alignment of the primary structure of the human and mouse invariant chain is presented. See text for detailed explanation.

thesis [6–8]. The region responsible for its self-association was mapped by analysis of deletion mutants of Ii to residues 163–183 [9,10]. NMR analysis of proteolytic fragments of Ii and of recombinant soluble Ii confirmed this result by identifying a highly structured, protease resistant luminal domain (aa 118–192) [11–13] as the trimerization domain (Fig. 1). This part of Ii is organized in an α-helical coiled-coil structure forming a scaffold suitable for simultaneous interaction with three αβ heterodimers [12]. Indirect evidence suggests that the transmembrane region might also contribute to stable homotrimer formation [14]. Moreover, a patch of hydrophilic residues (aa 47, 49 and 50) situated within the transmembrane domain of Ii near the luminal interface appears to be required for Ii trimerization [15].

2.2. Association with MHC II

Two disordered flexible domains (aa 72–110 and aa 187–216) flank the Ii trimerization domain (Fig. 1). The N-terminal unfolded Ii domain was described to acquire a significantly ordered structure upon complex formation with HLA-DR1 [16]. This domain contains the CLIP region (Fig. 1), which has been identified by means of various deletion and truncation mutants of Ii as necessary and sufficient for its interaction with MHC II [9,17]. This CLIP region corresponds to a nested set of peptides derived from Ii (aa 81–104) and eluted from MHC II of human and murine B cell lines defective for antigen presentation due to their lack of HLA-DM/H2-DM [18–20]. The determination of the crystal structure of a HLA-DR3–CLIP complex showed that CLIP

binds in the MHC II groove in a way highly resembling the binding of antigenic peptide, with residues 87–101 (core CLIP) occupying the groove and both extremities protruding out of the binding site [21].

Interestingly, Ii is able to bind to all MHC II allelic products despite important variations in their affinity for CLIP [22,23], suggesting that other regions than CLIP enforce the association of intact Ii with MHC II. Indeed, three additional luminal regions of Ii have been shown to contribute to the promiscuous binding of Ii to MHC II via contacts outside the polymorphic antigenic groove. They include a region immediately N-terminal of core CLIP (81–89) [24–27], a region C-terminal to core CLIP (103–118) [25,26,28] and a region located in the C-terminal trimerization domain (118–192) [13]. The two flanking regions could position core CLIP just above or in the peptide binding groove, thus providing the necessary means for efficient MHC II assembly. Indirect evidence suggested that further contacts between MHC II and Ii could take place within the transmembrane domains [14,29]. Finally, a recent study using peptide loaded MHC II, Ii truncation mutants and Ii/transferrin receptor (TfR) chimera indicated that Ii and MHC II transmembrane domains significantly contribute to the formation of αβIi complexes [30].

2.3. Chaperoning MHC II

Ii chain transiently associates with MHC II chains, favors their proper folding and therefore acts as a specific MHC II chaperone. Newly synthesized α and β subunits rapidly pass a transient stage, when they form high molecular mass aggregates, from which they are eventually rescued by Ii [31,32]. Individual pairs of αβ chains successively bind to a scaffold of an Ii trimer [6,33]. Association of the ER resident chaperone calnexin with each of the three subunits stabilizes the assembling complex until completion of the $(\alpha\beta Ii)_3$ nonamer [34,35]. Other chaperone molecules described to interact with MHC II chains in the ER include BiP (grp78) [31,36] and grp94 and Erp72 [37], but it is not established whether this constituted the physiological situation or was due to the absence of Ii in the studied cell types.

2.4. Prevention of premature peptide binding

The requirement of Ii for the formation of functional αβ dimers strongly depends on the MHC II allele considered [38,39] most likely because of differences in the intrinsic stability of αβ dimers [40]. Importantly, Ii association with MHC II prevents nascent MHC II to bind unfolded polypeptides or short peptides present in the ER. Binding of peptide and Ii were shown to be mutually exclusive in different experimental setups [33,41,42]. Nevertheless, endogenous antigen presentation by MHC II has been documented in some instances (for review see [3]). It has been recently established that presentation of an endogenous cytoplasmic antigen by MHC II requires proteasome processing and translocation into endosomes [43]. On the other hand, significant amounts of αβ dimers were found to stably bind a heterogeneous, but specific set of intact polypeptides of 20–200 kDa in the ER of Ii negative HeLa cells transfected with MHC II chains [44,45]. Interestingly, dendritic cells (DCs) from Ii deficient H-2^k mice also displayed mature MHC II complexes of varying molecular mass up to 120 kDa [39]. This phenomenon was not observed in B cells of Ii deficient mice [38], which could reflect cell type specific differences in the regulation of MHC II maturation (see below).

2.5. Egress from the ER

The study of Ii deficient mice allowed to establish

Table 1
Summary of Ii functions

Ii functions	Localization
Chaperone for MHC II α and β chains	ER
Inhibition of peptide binding to MHC II	ER, Golgi and endosomes
Egress of MHC II from the ER	ER/Golgi interface
Targeting to the endocytic pathway of MHC II	Golgi/EE interface
Endocytosis of αβIi complexes	Plasma membrane
Induction of large vacuoles/MVB	Endosomal pathway
Modulation of proteolytic enzymes	Endosomal pathway
Retention of αβIi and αβIi fragments	Endosomal pathway
Protection of H2-DM from degradation	Endosomal pathway
Promoting differentiation of immature B cells	Unknown

another important function of Ii, i.e. promoting efficient egress of MHC II from the ER. Ii helps MHC II to pass the ER quality control system, which allows only correctly assembled complexes to proceed to the Golgi. MHC II molecules from splenic B cells of Ii deficient mice exhibited defective post-ER transport resulting in reduced levels of MHC II surface expression, regardless of their capacity to assemble in the absence of Ii [38,46–48], demonstrating the role of Ii in MHC II transport out of the ER. Use of Ii truncation mutants and peptides (CLIP or antigenic peptides) covalently linked to the MHC II β chain [49,50] showed that occupancy of the MHC II binding groove in the ER was sufficient to promote efficient trafficking of nascent MHC II to the Golgi complex. However, this requirement also seemed to be cell type and allele dependent [39].

3. En route to the endocytic pathway

3.1. Targeting motifs

Another major function of Ii is to ensure the targeting of newly synthesized MHC II to the endocytic pathway, where the complexes can meet antigenic peptides. Transport steps which deviate from the constitutive secretory pathway require structural motifs present in the cytoplasmic tails of trafficking membrane proteins (for review see [51]). The signals mediating the sorting of αβIi complexes to the endocytic pathway were mapped to the N-terminal cytoplasmic domain of Ii [52–54]. Ii deleted of its cytoplasmic tail failed to dissociate from MHC II dimers and αβIi complexes were expressed at the plasma membrane [52,55]. By means of Ii mutants and chimera molecules, the endosomal localization signals were identified as two independent motifs containing two adjacent large hydrophobic non-aromatic residues at positions Leu7/Ile8 and Pro15/Met16/Leu17 in the Ii cytoplasmic tail [56–58] (see Fig. 1). NMR analysis of a synthetic peptide corresponding to residues 1–27 of human Ii indicated that the membrane distal motif is situated within a nascent helix, while the membrane proximal motif is part of a type 1 β turn [59]. Mutational analysis revealed the importance of the residues located in spatial proximity for efficient targeting to endosomes, suggesting that the motifs are recognized as part of a larger secondary structure [59,60]. The same signals also promote rapid internalization via clathrin coated pits of the plasma membrane [57,61]. By analyzing Ii-TfR chimeras in a quantitative internalization and transport to lysosomes assay Kang et al. reported that the two cytoplasmic di-leucine based signals were found important for lysosomal targeting. However, the Met16/Leu17 signal was less efficient for endocytosis [62]. This is consistent with results obtained in polarized MDCK cells, where basolateral sorting of Ii was mainly dependent on the membrane proximal motif in contrast to internalization, which was independently mediated by both signals [63]. A second study carried out in the same cell type mapped basolateral sorting information to both leucine based signals and to residues aa 20–30 of the cytoplasmic tail of Ii [64].

Several studies stressed the necessity of the Ii transmembrane domain for efficient delivery of MHC II complexes to the endocytic pathway [57,58]. Detailed mutational analysis of the Ii transmembrane domain identified two regions that were sufficient to confer lysosomal targeting on a reporter molecule without affecting its internalization rate [62]. Interestingly, the targeting function of Ii was found to depend on its capacity to form homotrimers [65,66]. Arneson et al. analyzed the intracellular transport of complexes formed by intact Ii and/or Ii mutants lacking the endosomal localization signals (Δ2–17) in L cell transfectants expressing MHC II. Their results established that a single Ii cytoplasmic domain present in a heterotrimer was sufficient to function as an internalization signal at the cell surface, but that homotrimerization of Ii was required for efficient targeting to endosomal compartments [65].

It has to be kept in mind that the sorting of MHC II molecules does not entirely depend on Ii. The cytoplasmic tails of both MHC II chains and especially a leucine based motif in the β chain are necessary for correct internalization and thus for recycling of mature MHC II [67–69]. However, the ultimate step of MHC II transport from lysosomes to the cell surface rather depends on stable peptide binding than on the cytosolic domains of the α and β chains [70].

Ii was further described to associate with the nonclassical MHC II molecule H2-DM/HLA-DM and to direct it to the endocytic compartments containing

MHC II [71–73]. H2-DM/HLA-DM are needed at this location to ensure efficient peptide loading onto MHC II. However, these non-classical MHC II molecules do not need Ii to reach their final destination. The cytoplasmic tail of the H2-DM and HLA-DM β chain contains a tyrosine based sorting signal, which ensures proper endosomal targeting and rapid internalization from the cell surface in the absence of Ii [72,73].

3.2. Which route, which entry door?

The actual route by which MHC II enter the endocytic pathway is an important determinant for their function in antigen presentation, as it influences the availability of different substrates for loading on MHC II and the environment for peptide binding, determining the nature of the material presented to T cells. Morphological and biochemical approaches indicate a direct transfer of MHC II molecules from the TGN to endocytic compartments in many different cell types [29,74–79]. However, surface expression of newly synthesized αβIi complexes followed by rapid internalization and access to the endosomal pathway has also been documented [61,80]. The importance of this indirect pathway depends very much on the activation state and cell type studied. In human DCs and monocytes the majority of newly synthesized MHC II complexes follow the itinerary via the cell surface [81].

The entry point of αβIi complexes into the endocytic pathway remained controversial for a long time. Several studies indicate that αβIi gain access to the endocytic pathway via early endosomes (EE)/sorting endosomes, since αβIi complexes were transiently detected in TfR positive EE before reaching late endocytic compartments [82–85]. Two recent studies showed by functionally ablating EE that this compartment was required for MHC II transport to peptide loading compartments [86,87]. Thus, regardless if nascent αβIi are directly sorted from the TGN to the endocytic pathway or via the cell surface, EE constitute the key compartment for the passage of all MHC II complexes.

3.3. Which transport machinery is recruited by Ii?

A related issue that remains controversial concerns the cytosolic machinery involved in the sorting of αβIi at the trans-Golgi network (TGN) level. Different cytosolic coat complexes are involved in TGN-endosomal sorting through their interaction with tyrosine or di-leucine based signals. AP-1 (and clathrin) would determine the transport of mannose 6-phosphate receptor (M6PR) to endosomes via di-leucine motifs [88], whereas AP-3 was proposed to mediate direct transport of lysosomal resident proteins to lysosomes [89]. Both represent potential candidates in mediating αβIi transport to endosomes.

Overexpression of Ii allowed the recruitment of AP-1 in a reconstituted cellular system in vitro [90]. Moreover, AP-1 and AP-2 were capable of interacting in vitro with the di-leucine based motifs of Ii cytoplasmic tail [91,92]. However, the capacity of Ii to bind and even to recruit AP-1 does not prove that αβIi are actually transported in AP-1 clathrin coated vesicles. Indeed, opposite conclusions were obtained, when the role of clathrin coated vesicles (CCVs) in MHC II trafficking was tested using a dominant negative mutant of clathrin, referred to as the hub fragment. Co-expression of hubs and αβIi complexes by transient transfection in mammalian cells caused cell surface accumulation of αβIi complexes [93], demonstrating that surface αβIi is endocytosed through CCVs. Similar conclusions were drawn from a study using a dominant negative mutant of dynamin [94]. Accumulation of hubs to inhibitory concentrations before induction of αβIi expression did not affect direct delivery of αβIi complexes to endosomes, indicating that this step occurred independently of CCVs, in contrast to the transport of HLA-DM to lysosomes, which was blocked in the same conditions [93]. B lymphoblasts derived from patients suffering from an enzymatic defect termed I-cell disease, possess a M6PR independent targeting pathway for lysosomal enzymes. Analysis of the transport of MHC II in such cells indicated that MHC II complexes can be transported from the TGN to the endocytic compartments in vesicles lacking clathrin and M6PR [95]. Murine and human B cells with selectively inactivated EE accumulated αβIi complexes in transport vesicles lacking both M6PR and AP-1, further suggesting that MHC II use an AP-1 independent transport pathway to reach endosomes [86]. The analysis of AP-1 deficient mice would have been a decisive tool to address this controversy but for the embry-

onic lethality of mice carrying a disruption of the μ1A- or γ-adaptin gene [96,97]. Analysis of MHC II trafficking and maturation in human and murine cells deficient for the AP-3 complex revealed no significant alteration in the kinetics of trafficking and maturation of MHC II, indicating that AP-3 is not essential for normal transport of MHC II [98,99].The recent description of a novel family of ubiquitous coat proteins termed GGAs, potentially involved in TGN to EE transport [100], opens the question of their possible involvement in the trafficking of MHC II.

Protein phosphorylation has been shown to positively regulate the recognition of leucine based endosomal targeting signals (see for instance [101]). Interestingly, in a wide variety of human antigen presenting cells (APCs), the Ii p35 isoform is phosphorylated on a serine residue in its cytosolic domain, probably by a member of the PKC family of serine/threonine kinases [102,103]. Stimulation of PKC activity by PMA significantly enhanced kinetics of Ii proteolysis and generation of SDS-stable MHC II complexes [104]. In addition, expression of an Ii-phosphorylation mutant resulted in impaired degradation of Ii and trafficking of MHC II [104]. In contrast, in mouse cells exposed to PMA, Ii degradation and MHC II maturation were enhanced and no phosphorylation of Iip33 was observed [105], possibly reflecting the absence of the Iip35 isoform in mouse. Finally, brefeldin A and aluminum fluoride affected the intracellular transport of MHC II, suggesting that ADP-ribosylation factor and heterotrimeric G proteins are involved in MHC II trafficking [79].

4. Within the endocytic pathway

4.1. Degradation events

Two degradation processes have to take place in a coordinated manner in the endocytic pathway to produce mature, peptide loaded MHC II. The Ii chain needs to be cleaved to provide peptide-receptive MHC II and antigenic peptides have to be produced by proteolysis of antigens. In the past years, numerous studies aimed at identifying the players involved in these proteolytic events.

Several proteases play a role in the stepwise degradation of Ii (reviewed in [106] [107]). First, Ii is cleaved to generate Iip22 (or LIP) and then Iip10 (or SLIP) by enzymes, which remain to be identified. Amongst them, the asparaginyl endopeptidase (AEP) is an attractive candidate for the first steps of Ii degradation as indirectly suggested by a recent study [108]. A cystatin homologue produced by a parasite was able to inhibit both AEP activity and lysosomal degradation of Ii. However, the involvement of AEP in the first steps of Ii degradation has yet to be formally demonstrated. The contributing enzymes are expected to be leupeptin insensitive, since cells exposed to this broad spectrum cysteine protease inhibitor tended to accumulate both Iip22 and Iip10, but not intact Ii. Biochemical and genetic evidence indicates that cathepsins B, D, S, L and F are not involved in the formation of Iip10 (see [109]). More information has been obtained regarding the conversion of Iip10 to CLIP. The major enzyme involved appears to be cathepsin S as demonstrated by the use of specific inhibitors and of knockout mice [110–114]. However, depending on the type of APC considered, other enzymes can also perform this conversion. In thymic epithelial cells, which do not express cathepsin S, production of CLIP was achieved by cathepsin L [115]. Moreover, macrophages from mice deficient for both cathepsins S and L appeared fully functional concerning Ii processing and peptide loading due to the action of cathepsin F [116].

Of note, very little information has been obtained about the degradation of antigen leading to the production of peptides within the endocytic pathway in vivo. Antigen processing would involve a thiol reductase activity, proteases with preferences for polar, charged or hydrophobic residues, as well as endo- and exo-peptidases (see [107]).

4.2. Modulation of proteolysis

Ii can modulate enzymatic activities and we will see later that the enzymatic activities driving Ii degradation can also be regulated. A non-covalent complex consisting of cathepsin L associated with an Ii fragment was isolated from human kidney [117]. This fragment proved to be a potent inhibitor of cathepsin L and originated from the alternatively spliced exon

6B of Iip41, one of the minor isoforms of Ii [118]. The in vivo significance of this interaction was recently revealed by analyzing bone marrow derived APCs from mutant mice that express either Iip31 or Iip41. Interestingly, Iip41 proved necessary for the expression and activity of mature cathepsin L indicating a chaperone function [119]. Modulation of cathepsin L activity by Iip41 would influence the production of functional MHC II, since this enzyme can convert αβp10 into αβCLIP in thymic epithelial cells [115]. Ii could further protect H2-DM from degradation, since its absence in splenocytes and mature DCs from Ii deficient mice resulted in strongly reduced levels of H2-DM [120]. This suggests that Ii could regulate peptide loading through its protective activity on H2-DM. Interestingly, immature DCs from Ii deficient mice contained normal levels of H2-DM [120], indicating that the changes induced within the endocytic pathway during DC maturation led to the exposure of H2-DM to a protease activity. How Ii would protect H2-DM and which enzyme(s) are involved remains to be established. Defects observed in the $CD4^+$ T cell repertoire of Ii deficient mice may thus reflect not only aberrant MHC II trafficking, but also compromised H2-DM functioning.

4.3. Remaining in the endocytic pathway

According to the emerging scenario, Ii associated to newly synthesized αβ chains would be degraded in a stepwise manner while the complexes progress deeper in the endocytic pathway to reach the loading compartments, where HLA-DM catalyzes the exchange of CLIP for antigenic peptides. The exact nature of these compartments has been a matter of intense investigations, but confusing conclusions (reviewed in [121]). It should be noted that trafficking of MHC II in the endocytic pathway appears highly dynamic and the classification of compartments is thus by definition artificial. It is clear nevertheless that peptide loading takes place in late endocytic compartments rich in MHC II (termed MIIC), which contain HLA-DM and certain proteases [121]. Ii and its fragments, Iip22 and Iip10, would drive associated αβ complexes to these MIICs.

The use of protease inhibitors, such as leupeptin, proved to be a great help; firstly, to demonstrate that Ii carries signals for retention within the endocytic pathway [122,123], secondly, to characterize and localize Ii degradation intermediates [83,124–126], and thirdly, to establish that degradation of Ii has to be complete in order to accomplish peptide loading and cell surface expression of mature MHC II [125,127]. Indeed, exposure to leupeptin led to a selective and dramatic accumulation of MHC II and Iip10 fragment in dense lysosomes [123–125]. The Iip10 fragment contains an intact cytoplasmic tail, carrying in all likelihood the retention signal constituted by the two di-leucine based motifs. In addition, results from a study performed with melanoma cells suggest that final degradation of Ii and peptide loading could take place in distinct compartments, but probably have to be coordinated in time [128].

4.4. Modifications of the endocytic pathway

Evidence has accumulated that expression of Ii chain modifies the morphology of endocytic compartments. Transfection of Ii chain into MHC II negative cell lines resulted in its accumulation in large vacuolar compartments containing late endosomal markers [10,58]. In the presence of MHC II morphological changes depended on overexpression of Ii chain relative to MHC II [84]. Inhibition of Ii degradation by various means created similar modifications of the endocytic pathway. Exposure of human EBV transformed B cells to concanamycin B, a highly selective inhibitor of the vacuolar H^+ (v)-ATPase responsible for acidification of endosomal compartments, resulted in inhibition of Ii chain degradation and striking accumulation of αβIi complexes in large and electron-lucent multivesicular bodies (MVB) [80]. Exposure of MelJuso cells to the protease inhibitor leupeptin caused an about 2-fold increase in total number of MVB enriched in Ii chain and Ii fragments [126]. In a MHC II expressing cell line deficient for HLA-DM expression, inefficient Ii chain proteolysis was observed, resulting in the accumulation of αβIi complexes in large, acidic intracellular compartments possessing unusual large vacuoles with internal membranes and vesicles [129]. In addition, transfection of HLA-DRα and β chains into embryonal kidney cells appeared sufficient to induce formation of typical MIIC compartments [130]. Therefore, Ii alone or together with MHC II

chains seems able to induce vesicle biogenesis within the endocytic pathway.

Interestingly, Ii also seems to delay traffic within the endocytic pathway. Prevention of Ii degradation induced a delay in transport of endocytosed material from early structures to late endosomes [126] but this could have resulted from a general effect of protease inhibitors. However, expression of α, β and wild type Ii in a fibroblast cell line led to a delayed transport of internalized peroxidase from early to late endosomes [131]. Similar observations were made in transfected COS cells [84]. In contrast, use of an Ii mutant lacking the cytoplasmic tail showed no effect, suggesting that Ii cytoplasmic tail could favor peptide loading by delaying access to lysosomes [131].

Therefore, Ii not only targets αβIi complexes from the TGN to EE and then retains them in deeper endocytic compartments, but may also modify general trafficking and morphology within this pathway. Ultimately, Ii would control the departure of MHC II from MIIC, since its total degradation is required for expression of MHC II at the cell surface [125].

5. Regulation of Ii functioning: the particular case of dendritic cells

Until a few years ago many cell biological studies on MHC II presentation had been achieved in EBV transformed B cells, a constantly activated cell type. This may have biased our view of the phenomenon, since MHC II presentation by primary cells may require induction by various means. Depending on the APC and the stimulus considered, these events might be differently regulated in situations such as DC maturation, antigen receptor mediated activation of B lymphocytes and exposure of monocytes/macrophages to inflammatory cytokines. The best documented examples of tight regulation of MHC II presentation come from studies on mouse and human DCs, which reveal interesting differences [132,133].

In human immature DCs, the majority of newly synthesized MHC II are first delivered to the cell surface in association with Ii and then rapidly internalized in the endocytic pathway [81]. There, degradation of Ii occurs in a cathepsin S dependent manner allowing MHC II to load peptides and access the cell surface [134]. αβ-Peptide complexes possess a half-life of about 10 h and are constantly and rapidly internalized and recycled [135]. Induction of DC maturation by inflammatory stimuli such as LPS, IL1β or TNFα results in many changes. A boost of MHC II synthesis is observed within 2 h [135], the activity of cathepsins S and B augments and the endosomal pH decreases [136]. This increases antigen degradation, SDS-stable dimer formation [136] and MHC II surface expression, while DC endocytic capacity is severely reduced [137]. Finally, the half-life of MHC II complexes increases by 10-fold favoring potential interaction with the antigen receptor of $CD4^+$ T cells at the plasma membrane [135]. Interestingly, exposure of immature DCs to IL10, an anti-inflammatory cytokine, diminished the levels of cathepsins S and B and prevented IL1β induced upregulation of these enzymes. This resulted in delayed MHC II maturation, inefficient antigen degradation and therefore in poor antigen presentation by MHC II [136], as initially observed in Langerhans cells [138]. Thus, when DCs are exposed to inflammatory stimuli, their capacity to induce an immune response would heavily depend on their cytokine environment.

More studies have been performed on MHC II trafficking in mouse DCs, generating sometimes conflicting conclusions resulting in part from the different setup used (various origins of the DCs expressing different MHC II allotypes). An attempt to summarize these results in a highly simplified manner is presented in Fig. 2. In immature mouse DCs newly synthesized MHC II were mainly observed in lysosomal compartments [139]. An elegant study using C4H3, a monoclonal antibody specific for $I\text{-}A^k$ associated with a peptide derived from the hen egg lysozyme (HEL) antigen, indicated that antigen presentation by MHC II did not take place in immature DCs, although internalized antigen and MHC II could extensively co-localize [140]. Exposure to inflammatory mediators such as LPS, CD40 ligand or TNFα induced a dramatic appearance of the $I\text{-}A^k$–HEL complexes at the cell surface [140,141]. DC maturation was accompanied by a boost of MHC II synthesis [142]. αβ-Peptide complexes acquired a longer half-life [139] and were transported together with costimulatory molecules via peripheral non-lysosomal vesicles to the cell surface [139,141]. There, they

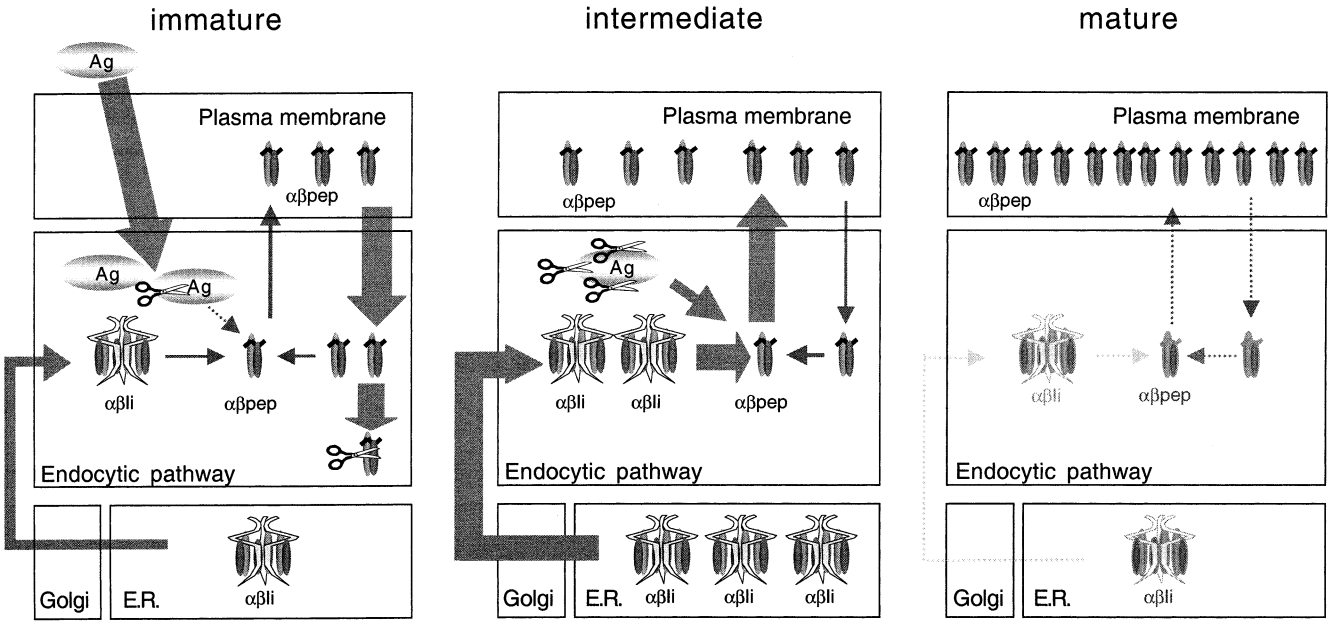

Fig. 2. Simplified model of MHC II trafficking in developing murine DCs. Three stages of DCs are presented. Immature DCs can be activated by inflammatory stimuli to become 'intermediate' DCs after a few hours, which differentiate into mature DCs after 24 h. The width of the arrows indicates the relative flow of molecules (MHC II or antigen). Faded symbols at the mature stage reflect the reduction of MHC II synthesis and trafficking. Ag stands for antigen, αβpep for MHC II complexes loaded with peptide. See text for detailed explanation.

stayed in clusters with co-stimulatory molecules and MHC I, potentially facilitating the formation of an immunological synapse between DCs and T cells [141]. These changes would result mostly from modifications in the degradation kinetics of Ii. From their study performed in H-2^b mice Pierre and Mellman proposed that Ii degradation is hampered in immature DCs and its p10 fragment remains associated with MHC II thus retained in lysosomal compartments [143]. This would result from cystatin C mediated inhibition of cathepsin S, the enzyme known to convert αβp10 into αβCLIP in DCs. Upon maturation, cystatin C activity decreases allowing final steps of Ii degradation and MHC II maturation to occur.

Cystatin C mediated inhibition of cathepsin S relies on a highly conserved region that binds the active site of the enzyme [144]. Cystatin C can also inhibit other cathepsins present in the endocytic pathway and contains a second inhibitory site specific for AEP [145]. Thus, expression of cystatin C in immature DCs could compromise the stepwise degradation of Ii and the peptide loading process. However, results obtained in DCs from cathepsin S deficient mice suggest that the proposed model is incomplete and that other mechanism(s) might be at work. Firstly, αβIip10 complexes were found not only in endocytic compartments but also at the cell surface [111–113], suggesting that retention by Iip10 was not complete. Secondly, when originating from an $H2^{k,q,s\ or\ u}$ background, normal maturation of I-A molecules occurred (see [106]), indicating that cathepsin S activity is dispensable. MHC II complexes appeared to behave normally in DCs from Ii knockout mice (H-2^k): they accumulated in MIIC of immature DCs, while they were exported to the plasma membrane during DC maturation. In addition, presentation of putative Ii dependent antigen by MHC II was not affected by the lack of Ii, suggesting that MHC II transport and peptide loading can occur in DCs in an Ii independent manner [39]. A recent study supports this conclusion and further indicates that mouse DCs may behave more like their human counterparts than initially thought [146]. Indeed, in immature DCs MHC II–peptide complexes had a short half-life as they were found to be re-endocytosed and degraded at a much faster rate than in mature DCs (see Fig. 2). Degradation of the Ii chain,

peptide loading and access of mature MHC II to the cell surface occurred at similar rates in immature and activated DCs [146]. Therefore, antigen presentation by DCs would be mainly controlled by the rate of re-endocytosis of MHC II–peptide complexes rather than by Ii chain degradation.

6. Additional function and future directions

Analysis of T independent responses in Ii deficient mice revealed that the development of their B lymphocytes was blocked at the immature stage. This blockage was independent of MHC II expression, since it was not observed in mice lacking MHC II β chain nor in mice deficient for the transactivator CIITA and correlated with levels of Ii [147]. Furthermore, expression of a truncated form of Ii (Ii1–82) essentially lacking the luminal domain was sufficient to drive differentiation of immature B cells in a process independent of Ii chaperone activity (I. Shachar and D. Matza, personal communication). Ii induces B cell maturation by activating a transcription pathway dependent on the transcription factor NF-κB [148]. More work is required to understand how Ii exerts this function.

Apart from its role on MHC II correct functioning and despite its small size, Ii represents a model protein of great interest for cell biological studies regarding chaperone function, intracellular protein sorting and degradation events. Many open questions still remain. The regulation of Ii and antigen degradation represents a field of intense investigations. The important role of cystatin C and cathepsin S in these processes is getting established. Note that cathepsin S was recently shown to regulate the activity of cathepsin L and the turnover of GILT (a thiol reductase involved in the first steps of antigenic degradation in endosomes [149]) in B lymphocytes [150]. The sorting machinery involved in Ii mediated targeting of MHC II complexes at the TGN level has yet to be identified. Similarly, the molecular basis of Ii influence on the biogenesis of multilamellar and vesicular compartments rich in MHC II remains elusive. No doubt investigations on the molecular basis of the functions of Ii will continue to produce information of great interest.

7. Note added in proof

A new function of the Ii chain has been recently documented, Ii would play a role in targeting CD1d molecules to the endocytic pathway (Jayawardena-Wolf et al., 2001 Immunity, 15 in press).

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